Factors Affecting the Susceptibility of the Beetle *Tribolium confusum* to Infection by *Hymenolepis diminuta*

Lorna C. Dunkley and D. F. Mettrick
Department of Zoology, University of Toronto, Canada

Received for Publication April 21, 1971

Abstract: The number of cysticercoids of the tapeworm *Hymenolepis diminuta* established in the insect intermediate host, *Tribolium confusum*, were found to be significantly influenced by the temperature at which the beetles were maintained, the number of days starvation of the beetles before infection, the period of exposure to the parasite eggs, and the age of the beetles.

The higher the temperature in the range 20°–35°C., the longer the period of starvation up to 8 days, the older the beetles up to 15 days, and double the period of exposure to the parasite eggs, all resulted in an increase in the average parasite burden per beetle.

In heavily parasitised hosts the effect of crowding resulted in smaller cysticercoids. Infectivity was not impaired.

INTRODUCTION

The adult tapeworm, *Hymenolepis diminuta* (Rudolphi, 1819), Blanchard, 1849 has been used extensively in experimental studies involving various aspects of the host-parasite relationship. In contrast, studies on the relationship between the larval stage of the tapeworm, and the intermediate host(s) are rare (Voge and Turner, 1956, Voge and Heyneman, 1957, 1958; MacDonald and Wilson, 1964; Kelly et al., 1967; Soltice et al., 1971).

The results regarding the factors affecting the infectivity of beetles to the larval stages of *H. diminuta*, are conflicting. Thus, while Kelly et al. (1967) reported that the age and the sex, but not starvation, of the beetle *Tribolium confusum* affected infectivity, Soltice et al. (1971) found that, in the two *Tribolium* species they were using *T. confusum* and *T. castaneum*, infection of the beetles was only influenced by temperature, and not by host species, host sex, or the number of eggs ingested. The present results confirm and delimit the effect of temperature upon beetle infectivity show that the number of eggs ingested does affect the level of beetle infection, and also show that the age of the beetle and starvation have significant effects upon the number of mature cysticercoids that are established per beetle.

MATERIALS AND METHODS

The initial strains of *Hymenolepis diminuta* and *Tribolium confusum* were obtained in 1965 from the Wellcome Laboratories for Experimental Parasitology (Professor C. A. Hopkins), University of Glasgow, Scotland.

The stock cultures of beetles were maintained at 26°C. on wholewheat flour. The adult worms were maintained in Wistar strain rats.

Unless stated otherwise, beetles, of varying age and sex were removed from several stock cultures, and pooled. From these pooled samples, groups of beetles were taken for individual experiments as required. The groups of beetles were starved for 4 days (96 hours), at 26°C. before exposure to the hymenolepid eggs.

Mature eggs from the gravid segments of *Hymenolepis diminuta* were concentrated in a balanced salt solution (Read et al., 1963). Two or three drops of this concentrate were added to a very small quantity of wholewheat flour on a filter paper in the bottom of a 2″ petri dish. The eggs and flour were thoroughly mixed prior to the introduction of the starved beetles. About 50 beetles were placed in each petri dish and allowed to feed for 24 hours at 26°C. before being transferred to culture jars filled with wholewheat flour to a depth of 1 inch. At this temperature development of the eggs to mature infective cysticercoids is completed in 13 days (Voge and Turner, 1956). Beetles were dissected for recovery of the cysticercoids on the 15th day postinfection, day 1 being the egg feeding period.

The results were evaluated statistically by analysis of variance (Snedecor and Cochran, 1967). Duncan's Multiple-Range Test (Steel & Torrie, 1960) was used to compare each group mean with every other within each analysis. Where shown in the tables, the results were calculated at the 95% level of significance. Included in the tables are the standard errors of the means.

RESULTS

Prior to determining the effect of age, starvation and temperature on the number of cysticercoids established per beetle, 5 groups of 50 beetles were infected and maintained as described above in Materials and Methods. From each group 20 beetles were picked at random on the 15th day postinfection, dissected, and the number of cysticercoids present in each beetle determined. The average number of cysticercoids per beetle in the five separate groups varied from 15.7 ± 2.2 to 20.0 ± 2.7. The overall mean was 17.5 ± 2.4, which may be considered as the standard value against which the following results may be compared.

1. AGE OF BEETLE: Young beetles, 24–48 hours old, were obtained from stock cultures, from which all adult beetles had been removed 24 hours previously. After pooling, the young beetles were divided into three groups. Group 1 was immediately starved for the mandatory 4 days, Group 2 was starved when the beetles were 6–7 days old, and Group 3 when the beetles were 11–12 days old. The three groups of beetles were thus 5–6, 10–11, and 15–16 days old respectively when allowed to feed on the hymenolepid eggs. Thirty beetles from each group were examined on the 15th day postinfection. All beetles were maintained at 26°C. throughout the experiment.

The results in Table 1 show that there was a statistically significant ($P < 0.05$) increase in the average number of cysticercoids recovered per beetle as the age of the beetles increased.

Table 1. Effect of age of beetle on the number of cysticercoids established per beetle.

Age of beetle (days)	5–6	10–11	15–16
Number of cysticercoids recovered per beetle ± S.E.	14.4 ± 1.7	16.4 ± 1.2	19.9 ± 1.7
Statistical significance*	|——————|		
		|——————|	

* Any two means not underscored by the same line are significantly different at the 95% level of significance.

2. STARVATION OF BEETLE: Beetles of random age were collected and divided into 8 groups as described in Materials and Methods. Each group was starved for a different length of time before the beetles were allowed to feed on the eggs. Thirty beetles from each group were examined on the 15th day postinfection. All beetles were maintained at 26°C. throughout the experiment.

There was a highly significant ($P < 0.001$) increase in the average number of cysticercoids established per beetle with an increase in the number of days that the beetles had been starved prior to infection (see Table 2).

3. TEMPERATURE: Prior to infection, 6 groups of pooled, random aged, beetles from stock cultures were acclimatised for 48 hours to the following six temperatures ± 0.5°C.: 20°, 23°, 26°, 29°, 32° and 35°C. After this period of acclimatisation all the beetle groups were maintained at their particular experimental temperature and starved for 4 days prior to exposure to hymenolepid eggs for 24 hours.

The average number of cysticercoids recovered per beetle on the 15th day postinfection for the six experimental temperatures, is shown in Table 3. Each value is the mean of 20 beetles.

Although some immature cysticercoids were noted, no cysticeroids had developed to maturity after a 26 day period at 20°C.

Table 2. Effect of starvation of beetle on the number of cysticercoids established per beetle.

Period of Starvation (days)	0	1	3	4	5	6	7	8
Number of cysticercoids recovered per beetle ± S.E.	7.7 ±1.3	8.1 ±1.5	8.5 ±1.6	10.3 ±1.4	12.7 ±1.6	13.1 ±1.6	14.4 ±1.6	14.9 ±1.5
Statistical significance*	|——————|							
		|——————|						
			|——————|					
				|——————|				

* Any two means not underscored by the same line are significantly different at the 95% level of significance.

TABLE 3. Effect of temperature on the number of cysticercoids established per beetle.

Temperature °C	20	23	26	29	32	35
Number of cysticercoids recovered per beetle ± S.E.	**0.0 ±0.0	8.0 ±1.2	15.5 ±1.7	17.1 ±2.0	19.4 ±2.1	24.2 ±2.7
Statistical significance*						

* Any two means not underscored by the same line are significantly different at the 95% level of significance.

** No mature cysticercoids were recovered at this temperature by 26 days postinfection.

4. LENGTH OF EXPOSURE TO HYMENOLEPID EGGS: In the above three experiments, beetles were allowed to feed on the hymenolepid eggs for 24 hours. To determine the effect of increasing this feeding period 50 beetles were starved for 7 days prior to exposure to the eggs. After 24 hours feeding, 25 of these beetles were transferred to a stock culture jar containing wholewheat flour, while the remaining 25 beetles were exposed to a second batch of hymenolepid eggs for a further 24 hours. The second beetle group was then transferred to a second culture jar containing flour. Twenty beetles from each group were examined for cysticercoids on the 15th day postinfection, day 1 being considered as the second 24 hour period in the case of the beetles exposed to the two batches of hymenolepid eggs.

There was a highly significant increase ($P < 0.01$) in the average number of cysticercoids established per beetle with an increase in the length of exposure of the beetles to the hymenolepid eggs. With 24 hours exposure the average number of cysticercoids recovered per beetle was 23.7 ± 2.2; with 48 hours exposure 34.4 ± 2.9 cysticercoids were recovered per beetle.

DISCUSSION

The results presented above show that the age of the host, the length of the period of starvation prior to feeding, the length of the egg feeding period and the temperature all had statistically significant effects on the number of cysticercoids of *H. diminuta* established per beetle. Of these 4 factors, the age of the beetles had the least influence on cysticercoid development; increasing the length of the egg feeding period had the greatest influence.

The results indicate that there is an increase in infectivity with increasing age of the beetles.

Kelly et al. (1967) found heavier infections of *H. diminuta* in older (23–24 weeks) male *Tribolium confusum* than in beetles only 4–5 weeks old but young female beetles were more heavily infected than older (23–24 weeks) female beetles. These authors also found that the level of infections declined in both male and female beetles that were 47–51 weeks old. It is unlikely, as suggested

by Kelly et al. (1967), that this decline was directly due to age immunity, as the beetles are sexually mature breeding adults before they are a month old. Rate of feeding may decline in old age. The increase in the number of cysticercoids recovered per beetle in the present experiments, may be a reflection of increased food intake associated with growth and sexual maturation in the beetles while they are still very young.

Similarly, the increase in the number of cysticercoids recovered per beetle due to an increase in the period of starvation prior to infection, may also be related to the level of food intake by the beetles. The moist egg-flour mixture was not as palatable to the beetles as their normal diet, and thus the degree of hunger of the animals probably regulated the amount of feeding on the egg-flour mixture. From a practical point of view 4 days starvation appeared to be the minimum period of starvation required before there was a significant increase in the number of cysticercoids recovered per beetle. The results from the fourth experiment, in which one group of beetles was allowed to feed on an egg-flour mixture for 24 hours and a second group for 48 hours, further supports the conclusion that the number of cysticercoids recovered per beetle was directly related to the amount of feeding carried out by the beetles. It is unlikely that, under the conditions tested, any chemical or mechanical factors could have significantly influenced the degree of infection of the beetles.

As an example of a mechanical factor it is pertinent to point out that Kelly et al. (1967) fed their beetles directly on tapeworm segments. Disregarding sex, the average number of cysticercoids recovered per beetle after 5–8 days starvation and up to 24 weeks old was only 6.9. In contrast, in our experiments in which the eggs were fed directly to the beetles after 5–8 days starvation (see Table 2), the average number of cysticercoids recovered per beetle was 13.8—a 100% increase. This difference may be simply because the beetles had to eat worm tissue before they even reached the eggs, and therefore, of course, ingested less eggs over the 24 hour feeding period.

Beetles which were heavily infected contained cysticercoids which showed the effects of crowding. The cysticercoids were smaller in size and their cercomeres were shorter than those of cysticercoids from light infections. No impairment in the infectivity of cysticercoids from heavy infections was noted when they were fed to the final host. Similar results due to crowding were recorded by Voge and Turner (1956) and Voge and Heyneman (1957). There was increased mortality among the heavily parasitised beetles, which confirms a similar observation of MacDonald and Wilson (1964).

Both Voge and Turner (1956) and Soltice et al. (1971) have shown that temperature significantly affects cysticercoid development and establishment. Voge and Turner (1956) reported that the most favourable temperature for raising cysticercoids of *H. diminuta* was 30°C. At this temperature development to maturity occurred in 8 days, as compared with 23 days at 20°C. In the

present experiments no mature cysticercoids were recovered from the beetles after 26 days at 20°C.; there was a steady increase in the number of mature cysticercoids recovered per beetle over the temperature range 23–35°C. Soltice et al. (1971) suggested that the effect of temperature on the intensity of infection was related to the efficiency of the enzyme-catalyzed reactions of the host and parasite which were concerned with the digestion of the egg layers and the penetration and development of the onchosphere.

Metabolic rates of poikilothermic animals are greatly increased with an increase in temperature. The increase in the number of mature cysticercoids recovered per beetle with increased temperature, may therefore be explained in terms of increased metabolic rates leading to increased food intake and thus a heavier infestation of the beetles.

It should also be noted that, contrary to the procedure used by Voge and Turner (1956) in their experiments on the effect of temperature, the beetles were acclimatised to the experimental temperatures before starvation and infection. Thus, temperature affected both infectivity of the beetles and development of the cysticercoids.

In conclusion, the four significant factors affecting the number of mature cysticercoids established per beetle may all be explained in terms of food intake by the beetles.

Literature Cited

KELLY, R. J., D. M. O'BRIAN AND F. F. KATZ. 1967. The incidence and burden of *Hymenolepis diminuta* cysticercoids as a function of the age of the intermediate host, *Tribolium confusum*. J. N. Y. Entomol. Soc. **75:** 19–23.

MACDONALD, I. G. AND P. A. G. WILSON. 1964. Host-parasite relations of the cysticercoid of *Hymenolepis diminuta*. Parasitol. **54:** 7 P.

VOGE, M. AND D. HEYNEMAN. 1957. Development of *Hymenolepis nana* and *Hymenolepis diminuta* (Cestoda; Hymenolepididae) in the intermediate host, *Tribolium confusum*. Univ. Calif. Pub. Zool. **59:** 549–580.

VOGE, M. AND D. HEYNEMAN. 1958. Effect of high temperature on the larval development of *Hymenolepis diminuta* (Cestoda: Cyclophyllidea). J. Parasitol. **44:** 249–260.

VOGE, M. AND J. A. TURNER. 1956. Effect of temperature of larval development of the cestode, *Hymenolepis diminuta*. Exp. Parasitol. **5:** 580–586.

READ, C. P., A. H. ROTHMAN AND J. E. SIMMONS. 1963. Studies on membrane transport with special reference to parasite-host integration. Ann. N. Y. Acad. Sci. **113:** 154–205.

SOLTICE, G. E., H. P. ARAI, AND E. SCHEINBERG. 1971. Host-parasite interactions of *Tribolium confusum* and *Tribolium castaneum* and *Hymenolepis diminuta*. Can. J. Zool. **49:** 265–273.

SNEDECOR, G. W. S. AND W. G. COCHRAN. 1967. Statistical Methods. 6th ed. Ames: Iowa State University Press.

STEEL, R. G. D. AND T. H. TORRIE. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. New York: McGraw Hill Book Co.